

Amendments to the Specification:

Please replace the paragraph at page 80, line 3 to page 81, line 13 with the following amended paragraph:

The *zlmda24* gene is located at the 7q21 region of chromosome 7. Several genes of known function map to this region that are linked to human disease. For example, an increase in the copy number of chromosome 7 is the most common chromosomal abnormality observed in human malignant gliomas (Bigner, S.H. et al., Cancer Res. 48:405-411, 1998; Bigner, S.H. et al., J. Neuropathol. Exp. Neurol. 47:191-205, 1998; Bigner, S.H. et al., Atlas Sci. Biochem. 333-336, 1998). Thus, since the *zlmda24* gene maps to chromosome 7, the *zlmda24* polynucleotide probes of the present invention can be used to detect chromosome 7 trisomy and other chromosome 7 gains, and particularly chromosome 7q21 chromosome gain associated with human malignant gliomas. Hence, the polynucleotides of the present invention can serve as a diagnostic for human malignant gliomas. Moreover, trisomy of chromosome 7 is often found in papillary renal carcinoma tumors and the increase in copy number of mutated MET proto-oncogene (7q31) is believed to be a factor in tumorigenesis (Zhuang, Z. et al., Nature Genet. 20:66-69, 1998). Thus, the *zlmda24* polynucleotide probes of the present invention can be used to detect chromosome 7 trisomy associated with papillary renal carcinoma tumors. Hence, the polynucleotides of the present invention can serve as a diagnostic for papillary renal carcinoma tumors. Moreover, several chromosomal aberrations at 7q21 including deletions, rearrangements, and chromosomal breakpoints, and translocations are seen in humans with ectrodactyly, for instance seen in split hand/foot malformation (SHFM1). Moreover, the critical region for ectrodactyly is the 7q21.1-7q22.1 locus of chromosome 7. Thus, since the *zlmda24* gene maps to this critical region, the *zlmda24* polynucleotide probes of the present invention can be used to detect chromosome deletions, translocations and rearrangements associated with ectrodactyly. Moreover, chromosomal deletions of 7q21.1-q22 are associated with mucopolysaccharidosis type VII (MPS VII), which is manifested by mental retardation, amongst other phenotypic abnormalities. Similarly, *zlmda24* polynucleotide probes of the present invention can be used to detect chromosome deletions, translocations and rearrangements associated with MPS VII. Moreover, the multi drug resistance 3 gene (*MDR3*) gene, mutations that confer drug resistance to hydrophobic drugs, e.g., chemotherapeutics, maps to 7q21.1. Moreover, amongst other genetic loci, those for Cholistasis (7q21.1), collagen abnormalities including maternal disomy (i.e., isodisomy) of chromosome 7 (7q22.1) and deletions (17q21-q22), frontonasal dysplasia (7q21), Zerwilliger syndrome (7q21-q22), and cerebral cavernous malformations (7q11-q21) all manifest themselves in human disease states as well as map to this region of the human

genome. See the Online Mendellian Inheritance of Man (OMIM™, National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD) gene map, and references therein, for this region of human chromosome 7 on a publicly available world wide web server (~~(http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/getmap?chromosome=7q21)~~ (www3.ncbi.nlm.nih.gov/htbin-post/Omim/getmap?chromosome=7q21)). All of these serve as possible candidate genes for an inheritable disease that show linkage to the same chromosomal region as the zlmda24 gene. Thus, zlmda24 polynucleotide probes can be used to detect abnormalities or genotypes associated with these defects.

Please replace the paragraph at page 89, lines 15-22 with the following amended paragraph:

Scanning of a translated murine cDNA database against human zlmda24 resulted in identification of an expressed sequence tag (EST) sequence (EST3295730). A mouse probe, based on the identified EST was generated by PCR using oligos ZC29,714 (SEQ ID NO:7) and ZC29,984 (SEQ ID NO:8), and in-house mouse testis library as a template under the following reaction conditions: 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 65°C for 30 seconds, 72°C for 30 seconds; followed by 72°C for 7 minutes. The PCR fragment was gel purified using ~~QIAquick gel extraction kit (Qiagen)~~ a gel extraction kit (QIAQUICK kit; QIAGEN).

Please replace the paragraph at page 89, lines 23 to page 90, line 4 with the following amended paragraph:

The mouse testis library was an arrayed library representing 9.6×10^5 clones. The library was screened using the PCR conditions described above. The library was deconvoluted down to a positive pool of 250 colonies. The positive mouse testis pool was plated and filter-lifted using ~~Hybond-N filters (Amersham, England)~~ membrane filters (HYBOND-N; Amersham, England). A total of about 1000 colonies were screened on 4 filters lifted from plates of about 250 colonies per plate. The filters were marked with a hot needle for orientation, then denatured for 6 minutes in 0.5 M NaOH and 1.5 M Tris-HCL pH 7.2. The filters were then neutralized in 1.5 M NaCl and 0.5 M Tris-HCL pH 7.2 for 6 minutes. The DNA was affixed to the filters using a ~~Stratalinker~~ UV crosslinker (STRATALINKER; Stratagene, La Jolla, Ca.) at 1200 joules. The filters were prewashed at 65 degrees C in prewash buffer consisting of 0.25X SSC, 0.25% SDS and 1mM EDTA. The solution was changed a total of three times over a 45-minute period to remove cell debris. Filters were prehybridized overnight at 65°C in 25ml ~~Expresshyb~~ hybridization solution (EXPRESSHYB; Clontech, Palo Alto, Ca.).

Please replace the paragraph at page 90, lines 5-14 with the following amended paragraph:

The probe was generated by PCR using oligos ZC29714 (SEQ ID NO:7) and ZC29984 (SEQ ID NO:8), as described above. The PCR fragment was gel purified using ~~QIAquick~~ a gel extraction kit (QIAQUICK; QIAGEN, Qiagen, Santa Clarita, Ca.). The probe was radioactively labeled with ^{32}P using ~~the Rediprime II DNA Labeling a labeling~~ system (REDIPRIME II; Amersham, UK) according to Manufacturer's specifications. The probe was purified using a ~~Nuetrap~~ push column (~~Stratagene cloning system~~ NUCTRAP; Stratagene Cloning Systems, La Jolla, Ca.). ~~Expresshyb (Clontech, Palo Alto, Ca)~~ Commercially available hybridizing solution (EXPRESSHYB; Clontech, Palo Alto, Ca) was used ~~for the hybridizing solution for the filters~~. Hybridization took place overnight at 65°C. Filters were rinsed 2X in 65°C in pre-wash buffer (0.25X SSC, 0.25% SDS and 1mM EDTA). Then the filters were washed 2X in pre-wash solution at 65°C. Filters were exposed to film for 1 days at -80°C.

Please replace the paragraph at page 90, line 25 to page 91, line 21 with the following amended paragraph:

A panel of cDNAs from human tissues was screened for zlmda24 expression using PCR. The panel was made in-house and contained 94 marathon cDNA and cDNA samples from various normal and cancerous human tissues and cell lines and is shown in Table 5, below. The cDNAs came from in-house libraries or marathon cDNAs from in-house RNA preps, Clontech RNA, or Invitrogen RNA. The marathon cDNAs were made using the marathon-ReadyTM kit (Clontech, Palo Alto, CA) and QC tested with clathrin primers ZC21195 (SEQ ID NO:9) and ZC21196 (SEQ ID NO:10) and then diluted based on the intensity of the clathrin band. To assure quality of the panel samples, three tests for quality control (QC) were run: (1) To assess the RNA quality used for the libraries, the in-house cDNAs were tested for average insert size by PCR with vector oligos that were specific for the vector sequences for an individual cDNA library; (2) Standardization of the concentration of the cDNA in panel samples was achieved using standard PCR methods to amplify full length alpha tubulin or G3PDH cDNA using a 5' vector oligo ZC14,063 (SEQ ID NO:11) and 3' alpha tubulin specific oligo primer ZC17,574 (SEQ ID NO:12) or 3' G3PDH specific oligo primer ZC17,600 (SEQ ID NO:13); and (3) a sample was sent to sequencing to check for possible ribosomal or mitochondrial DNA contamination. The panel was set up in a 96-well format that included a human genomic DNA (Clontech, Palo Alto, CA) positive control sample. Each well contained approximately 0.2-100 pg/μl of cDNA. The PCR reactions

were set up using oligos ZC28587 (SEQ ID NO:14) and ZC28591 (SEQ ID NO:15), Advantage TaqTM (Clontech), and RediLoad REDILOAD dye (Research Genetics, Inc., Huntsville, AL). The amplification was carried out as follows: 1 cycle at 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 60.0°C for 30 seconds and 72°C for 30 seconds, followed by 1 cycle at 72°C for 10 minutes. About 10 µl of the PCR reaction product was subjected to standard ~~Agarose~~ agarose gel electrophoresis using a 4% agarose gel. The correct predicted DNA fragment size was primarily observed in testis (i.e., all the testis samples on plate), and very weakly in salivary gland. The other tissues and cell lines tested were negative, indicating that the expression of zlmada24 is testis-specific.

Please replace the paragraph at page 93, lines 4-11 with the following amended paragraph:

Zlmada24 was mapped to chromosome 7 using the commercially available "GeneBridge 4 Radiation Hybrid (RH) Mapping Panel" (Research Genetics, Inc., Huntsville, AL). The GeneBridge 4 RH panel contains DNA from each of 93 radiation hybrid clones, plus two control DNAs (the HFL donor and the A23 recipient). A publicly available WWW server (~~<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>~~) (www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl) allows mapping relative to the Whitehead Institute/MIT Center for Genome Research's radiation hybrid map of the human genome (the "WICGR" radiation hybrid map) which was constructed with the GeneBridge 4 RH panel.

Please replace the paragraph at page 93, line 12 to page 94, line 5 with the following amended paragraph:

For the mapping of Zlmada24 with the GeneBridge 4 RH panel, 20 µl reactions were set up in a 96-well microtiter plate compatible for PCR (Stratagene, La Jolla, CA) and used in a ~~"RoboCycler Gradient 96"~~ thermal cycler (ROBOCYCLER GRADIENT 96; Stratagene). Each of the 95 PCR reactions consisted of 2 µl 10X PCR reaction buffer (Qiagen, Inc., Valencia, CA), 1.6 µl dNTPs mix (2.5 mM each, PERKIN-ELMER, Foster City, CA), 1 µl sense primer, ZC 29,567 (SEQ ID NO:16), 1 µl antisense primer, ZC 29,568 (SEQ ID NO:17), 2 µl ~~"RediLoad"~~ dye (REDILOAD; Research Genetics, Inc., Huntsville, AL), 0.1 µl ~~Qiagen HotStarTaq DNA Polymerase~~ DNA polymerase (HOTSTARTAQ; QIAGEN; 5 units/µl), 25 ng of DNA from an individual hybrid clone or control and distilled water for a total volume of 20 µl. The reactions were overlaid with an equal amount of mineral oil and sealed. The PCR cycler conditions were as follows: an initial 1 cycle 15 minute denaturation at 95°C, 35 cycles

of a 1 minute denaturation at 95°C, 1 minute annealing at 48°C and 1 minute and 15 seconds extension at 72°C, followed by a final 1 cycle extension of 7 minutes at 72°C. The reactions were separated by electrophoresis on a 2% agarose gel (EM Science, Gibbstown, NJ) and visualized by staining with ethidium bromide.

Please replace the paragraph at page 94, line 19 to page 95, line 14 with the following amended paragraph:

A zlmda24 fragment containing BamHI and XbaI restriction sites on the 5' and 3' ends, respectively, was generated by PCR amplification from a plasmid containing zlmda24 cDNA (Example 1) using primers ZC29,055 (SEQ ID NO:19) and ZC29,056 (SEQ ID NO:20). Due to the method of cloning, there are two introduced amino acids (Ala-Leu) prior to the Glu-Glu tag (SEQ ID NO:18) that come from the Xba cloning site. The PCR reaction conditions were as follows: 20 cycles of 94°C for 30 seconds, 77°C for 60 seconds; 1 cycle at 72°C for 7 min; followed by 4°C soak. The fragment was visualized by gel electrophoresis (1% ~~SeaPlaque~~1% ~~NuSieve~~ SEAPLAQUE/1% NUSIEVE). The band was excised and purified using a ~~Qiagen-Gel Extraction Kit~~ gel extraction kit (QIAGEN) according to the manufacturer's instructions. The purified fragment was then subcloned into a PCR 2.1 TOPO vector (Invitrogen) according to the manufacturer's instructions. A subclone with the correct zlmda24 sequence was identified by sequencing. Plasmid DNA from the correct subclone was digested with BamHI and Xba I and ligated into an BamHI/XbaI digested baculovirus expression vector, pZBV32L. The pZBV32L vector is a modification of the pFastBac1™ (Life Technologies) expression vector, where the polyhedron promoter has been removed and replaced with the late activating Basic Protein Promoter, and the coding sequence for the Glu-Glu tag as well as a stop signal is inserted at the 3' end of the multiple cloning region). About 25 nanograms of the restriction digested zlmda24 insert and about 32 ng of the corresponding vector were ligated overnight at 16°C. The ligation mix was diluted 3 fold in TE (10 mM Tris-HCl, pH 7.5 and 1 mM EDTA) and 4 fmol of the diluted ligation mix was transformed into DH10b electrocompetent cells (Life Technologies) according to manufacturer's direction. The transformed DNA and cells were diluted in 450 µl of SOC media (2% ~~Bacto~~ BACTO Tryptone, 0.5% ~~Bacto~~ BACTO Yeast Extract, 10 ml 1M NaCl, 1.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄ and 20 mM glucose) and plated onto LB plates containing 100 µg/ml ampicillin. Clones were analyzed by restriction digests. The zlmda24-CEE polypeptide is shown in SEQ ID NO:23.

Please replace the paragraph at page 103, line 24 to page 104, line 5 with the following amended paragraph:

Oligonucleotide features corresponding to zlmada24 were found on the 'Transcription_35' (or TXN35) microarray experiments (Cho, RJ et al. Nat. Genet. 27:48-54, 2001). In these experiments Cho et al. employed a single-color approach to examine the expression patterns of primary fibroblasts over the course of 24 hours in the cell cycle. The cells were initially synchronized using a double thymidine-block protocol. These experiments were designed to identify novel genes involved in cell cycling and provide information on the function of previously uncharacterized transcripts. The Transcription_35 data set is from experiments employing Affymetrix 35,000 human gene oligonucleotide arrays (Affymetrix, Inc., Santa Clara, CA). These experiments were conducted only one time for each (2 hour) time point. See, Cho et al. *supra.* for details of all these experiments. The resulting data were obtained from the public Web site through The Salk Institute, La Jolla, CA microarray chip data (~~<http://www.salk.edu/docs/labs/chipdata/primary.html>~~) (www.salk.edu/docs/labs/chipdata/primary.html).